AWARD NUMBER: W81XWH-15-1-0118

TITLE: Antimicrobial Silk Ocular Drug Delivery Implant for Chronic Posterior Segment Diseases

PRINCIPAL INVESTIGATOR: Kaplan, David

CONTRACTING ORGANIZATION: Tufts University
Boston, MA 02111

REPORT DATE: June 2016
~~October 2016~~

TYPE OF REPORT: Annual Technical Progress Report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ORGANIZATION.

15May2015 - 14May2016

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
June2016 ~~01/09/2016~~	Annual Technical Progress Report	~~May2015~~ ~~August2016~~

4. TITLE AND SUBTITLE
Antimicrobial Silk Ocular Drug Delivery Implant for Chronic Posterior Segment Diseases

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-15-1-0118

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
David L, Kaplan, Irmgard Behlau

email: david.kaplan@tufts.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Tufts University
4 Colby Street
Medford, MA 02155

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
US Army Medical Research and Materiel Command
Fort Detrick, MD 21702

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for Public Release, Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
An intraocular insert based on silk fibroin was designed and optimized. The insert contains a hollow channel that was preloaded with the anti-VEGF antibody bevacizumab. The device was covalently coated with the antibacterial polymer HMPEI to protect against biofilm formation and the transmission of harmful bacteria to the interior of the ocular chamber. The device is fist of its kind to be designed for sustained release of a large-molecule drug into the vitreous humor for the prevention of posterior segment diseases such as diabetic retinopathy and age-related macular degeneration. An in vitro model and an in vivo rabbit eye model were developed for determining the release profile of the therapeutic antibody from the device. Through the combination of the aqueous processing of a naturally occurring biodegradable protein (fibroin) into the device mold, antibacterial chemical modification of the device exterior, and the optimization of the delivery medium, the present system offers a compelling alternative to the standard-of-care for the treatment of posterior segment diseases.

15. SUBJECT TERMS
Silk, drug delivery, intraocular implant, eye, diabetes, chronic posterior segment diseases, antimicrobial, ocular, sustained release

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	11	19b. TELEPHONE NUMBER *(Include area code)*

Table of Contents

Page

Introduction:

Posterior segment diseases, e.g., diabetic retinopathy and age-related macular degeneration, are a leading cause for the loss of sharp visual acuity for patients in the U.S. and worldwide, especially in the elderly population. Most of these diseases are associated with the formation of aberrant blood vessels in the posterior segment, especially in the macula. A promising treatment for tackling some of these diseases involves the release of an anti-VEGF antibody, such as bevacizumab, into the vitreous humor to prevent the formation of such blood vessels. For the present project, a protein-based biodegradable drug-delivery system was developed for the sustained release of bevacizumab into the vitreous humor. The system comprises an intraocular insert with antibacterial coating with the drug loaded in a hollow channel within. The design of the device was optimized and a protocol for the surgical insertion of the device inti rabbit eyes was developed. The device was proven to be biocompatible and mechanically robust.

Keywords

Silk; fibroin; diabetic retinopathy; macular degeneration; HMPEI; rabbit model; bevacizumab.

Accomplishments

The major goals for the projects as stated in the SOW were: (a) "design of a refillable and long-term intraocular silk-based device for the delivery of anti-VEGF drugs to the posterior eye", (b) "Silk Drug Delivery Implant Design", (c) "In vitro Silk-based anti-VEGF Drug Delivery and Pharmacokinetic Studies", (d) "Development of an antimicrobial, N,N-hexyl, methyl-polyethylenimine (HMPEI) derivatized intraocular silk drug delivery implant", (e) "Maximization of HMPEI-bound silk surface area, host cell biocompatibility, and antimicrobial efficacy", (f) "HMPEI-silk implant mechanical stability and in vitro drug release studies", (g) "Assessment of the anti-VEGF HMPEI-silk drug delivery implant system in the rabbit in vivo", (h) "Assessment of the anti-VEGF HMPEI-silk drug delivery implant system in normal pigmented rabbit models in vivo", and (i) "Assessment of the anti-VEGF HMPEI-silk drug delivery implant system in a diabetic retinopathy rabbit models in vivo". In the following paragraphs, our progress towards these goals are described.

1. After numerous iterations, we developed the format of the intraocular implant (a silk-fibroin-based hollow gel-spun tube) and the format of the drug-delivery medium (a silk fibroin based lyophilized hydrogel). **Figure 1** depicts the dimensions and the make-up of the device. Spinning a concentrated 15 % (w/v) silk solution over a steel wire (diameter = 500 μm), then lyophilizing it yielded a silk tube with consistent inner diameter. Then the silk tubes (still wrapped around the steel wire) were submerged in methanol for 1 h to induce β-sheet formation. Then the tubes were separated from the wire by sliding the tubes over the wire. The gel-spun tube was then cut into smaller pieces (5 mm in length). Although the inner diameter of the device is fixed by the outer diameter of the steel wire, the outer shape of the device is determined by the deposition pattern of silk solution. Instead of a uniform outer surface, we chose to prepare a peanut-shaped device for ease of placing the device into the target organ. The device is based on a single format and has thus no junctions that may affect the mechanical integrity. The device has a consistent and well-defined delivery channel for storing the drug-releasing material.

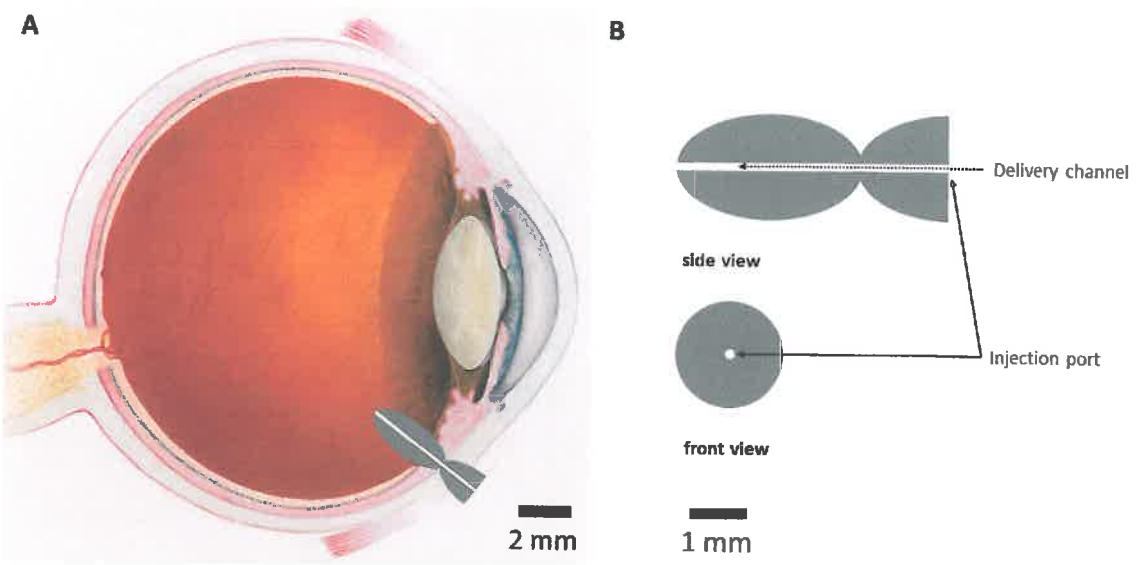

Figure 1. Cartoon depiction of the silk-based intraocular device. (A) The placement of the device into the intraocular space through *pars plana*. The graphic of the eye has been adapted from an image by National Eye Institute: https://www.flickr.com/photos/nationaleyeinstitute/7544656020/in/album-72157646829197286/ (Creative Commons 2.0 license). (B) Detailed view of the device (4-5 mm long) from side and front.

2. Silk fibroin in the device was successfully functionalized covalently with the antimicrobial polymer N,N-hexyl,methyl-polyethylenimine (HMPEI) in three steps. The chemical reactions involved are depicted in **Figure 2**. Briefly, the devices were placed in borate buffer at 0°C and diazonium salt solution (prepared from 4-aminobenzoic acid, p-toluenesulfonic acid and sodium nitrite solution) was added to it. The samples turned bright orange. The samples were washed with distilled water and air-dried. Next, the devices were placed in a solution of 217 kDa polyethylenimine (PEI) in aqueous dimethylformamide. We then added 1-hydroxy-benzotriazole (HOBT) and N-(3-dimethylamino)propyl-N'-ethylcarbodiimide (EDC) to promote the formation of amide linkage between the polymer and the sponge surface tyrosine residues. The resulted sponges were washed and reacted with a mixture of 1-bromohexane, trimethylamine, and t-amyl alcohol. Finally, the samples were washed and added to a mixture of t-amyl alcohol and iodomethane to complete the formation of positively charged quaternary ammonium groups in the polymer chains, resulting in HMPEI-functionalized azo-silks. No difference in color was observed between the azo-silk and HMPEI-azo-silk devices (both were bright orange). A few photographs of the device are shown in **Figure 3**.

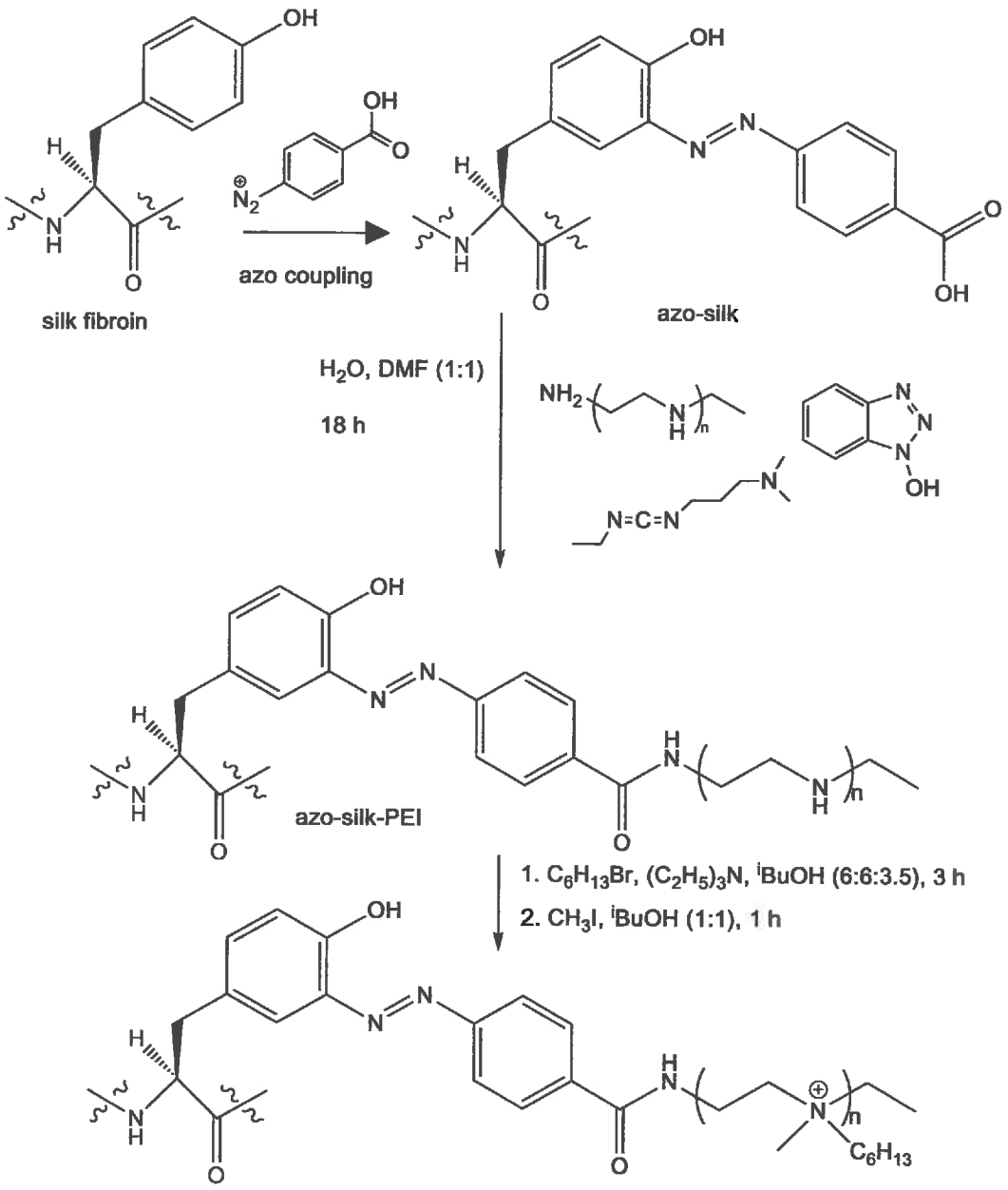

Figure 2. Three-step chemical functionalization of the silk fibroin-based device. The functionalization takes place on the exposed tyrosine residues in the silk protein.

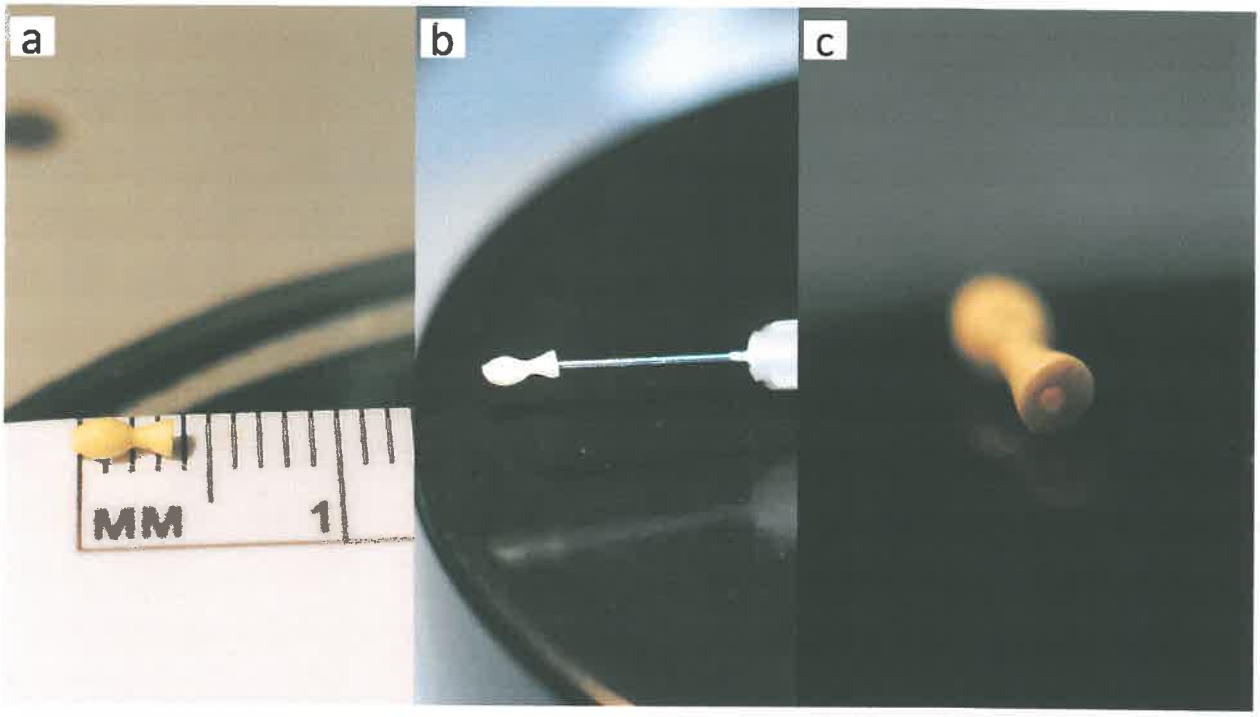

Figure 3. Photographs of the silk-based device after chemical functionalization and before loading the anti-VEGF drug, bevacizumab.

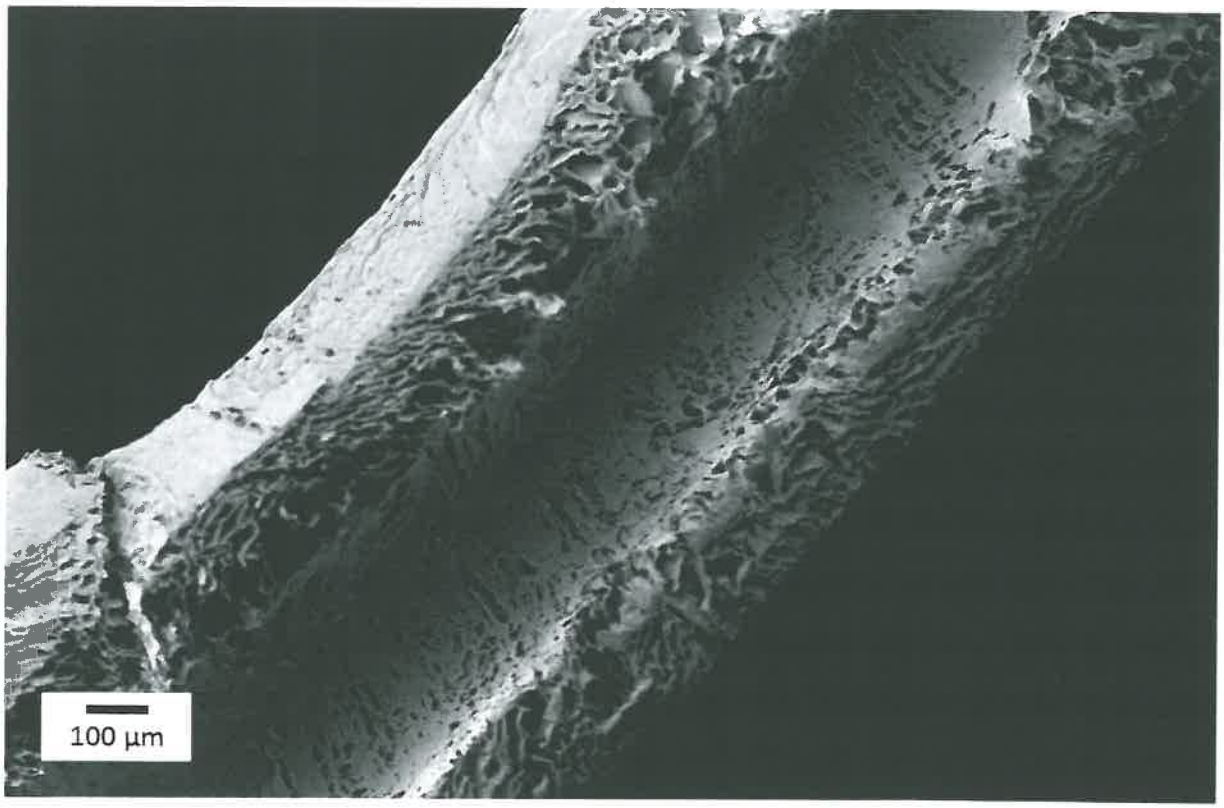

Figure 4. SEM image of the vertical section of the device, showing the inner channel.

3. By scanning electron microscopy (SEM), the change in morphology of the device surface and interior channel in response to the covalent chemical functionalization was investigated (**Figure 4**). The changes in device shape, channel width, and porous structure were minimal.

4. The anti-VEGF antibody, bevacizumab, was successfully incorporated into the inner channel of the hollow device in a lyophilized hydrogel (lyogel) format. First, we pipetted out 7 mL of 4.8 % in vivo grade silk fibroin solution in a 50 mL falcon tube. The silk fibroin solution was then sonicated for 20 seconds. The solution remains fluid. Then 1 mL of the sonicated solution was pipetted into a 10 mL tube and lyophilized powder of bevacizumab was added such that the final concentration of bevacizumab in the silk solution was 3.75 % (w/v). The solution was mixed by pipetting up and down, until no drug powder was visible. The solution was poured onto a petri dish and the devices were dipped into the solution in an upright fashion, such that the drug-loaded solution entered the device channel by capillary action. The sonicated solution of fibroin formed a hydrogel over time (approximately 2 hours) which could be observed in 6 mL of excess silk solution that was not used for dissolving the bevacizumab. After 2 hours, the devices were put in a freezer for 3 hours and then lyophilized overnight. A separate batch of sonicated silk solution was prepared and the ends of the devices were dipped in the solution to create hydrogel plugs, thereby sealing the device. The drug-loaded devices were treated with hydrogen peroxide gas plasma for disinfection.

5. A sterilization protocol for the drug-loaded device was finalized (plasma treatment).

6. An *in vitro* drug release study was carried out from the silk lyogel into a physiologically relevant buffer (1X DPBS) at physiological temperature (37 °C).

7. An *ex vivo* implantation of the functionalized device into pig eyes was performed to determine the optimal surgical protocol to insert the device into the intraocular vitreous.

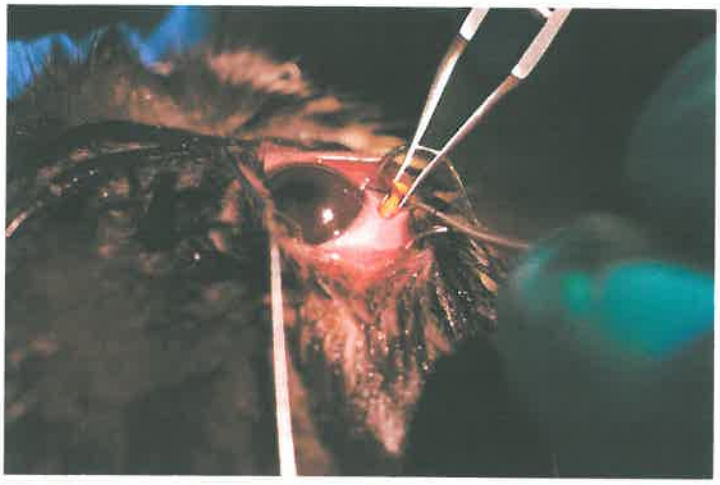

Figure 5. Surgical implantation of the drug-loaded device into rabbit eyes.

Figure 6. Use of the conjunctival flap to cover the outer surface of the device, further reducing the exposure of the device to microbes at the surface of the eye.

8. The antibody-loaded device was surgically implanted into rabbit eyes through pars plana (**Figure 5**). A conjunctival flap was used to minimize the outward exposure of the device (**Figure 6**).

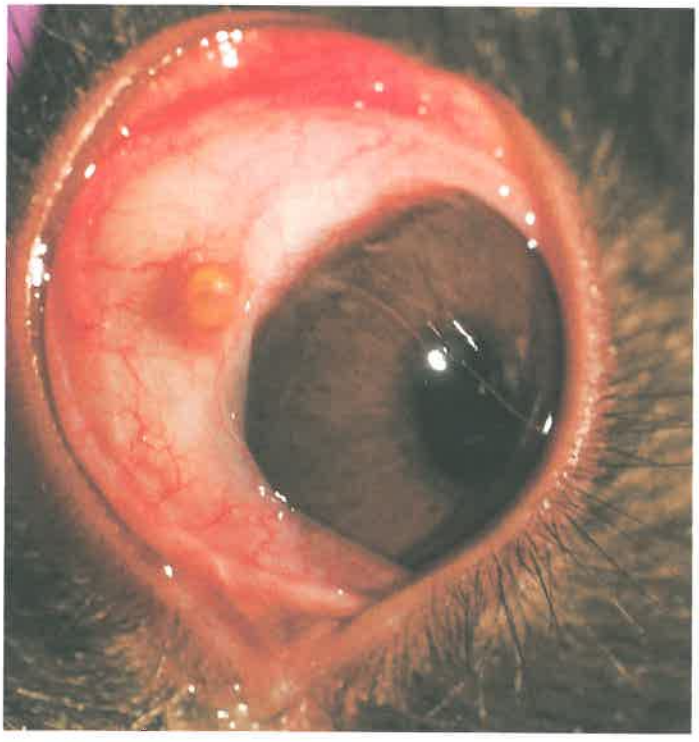

Figure 7. A rabbit eye post-implantation. No sign of inflammation was observed. The yellow color of the device and the hollow inner delivery channel is apparent through the conjunctival flap.

9. Short-term (1 month) medical observation suggests that the device is biocompatible and no sign of bacterial biofilm formation was observed on the implanted device (**Figure 7**).

The current project was crucial for the professional development of Dr. Biplab Sarkar (postdoctoral training, gained expertise in design and antibacterial functionalization of drug-delivery devices) and Dr. Irmgard Behlau (presentation at a conference).

Detailed pharmacokinetic study of in vivo drug release will be reported in the next report.

Impact

The device could be a relatively easy way to administer the anti-VEGF drug into the vitreous humor of the patients and could alleviate the need for repeated injection schedule. Thus, the drug-delivery system could prevent the worsening of visual acuity for patients with posterior segment diseases. The design of the device could also be used in future as a template for the development of medical inserts/constructs with antibacterial coating. We have nothing to report at this stage on the impact on technology transfer and the impact on society beyond science and technology.

Changes/Problems

The device design was altered from the initial version proposed in the grant for ease of manufacturing as well as to improve the handling properties.

Products

Conference presentation: "Antimicrobial Silk Ocular Drug Delivery Implant for Chronic Posterior Segment Diseases", Ocular Microbiology and Immunology Group (OMIG) Meeting, October 14, 2016; Chicago, IL.

Techniques: non-uniform gel-spinning of silk fibroin, covalent antibacterial functionalization of silk fibroin.

Participants and Other Collaborating Organizations

Name:	*Biplab Sarkar*
Project Role:	*Postdoctoral Scholar*
Researcher Identifier (e.g. ORCID ID):	*orcid.org/0000-0002-9298-2392*
Nearest person month worked:	*2*
Contribution to Project:	*Dr. Sarkar contributed towards the design and chemical functionalization of the device, optimization of the drug-delivery medium, as well as the determination of the drug release profile.*
Funding Support:	*This grant*

Name:	*Irmgard Behlau*
Project Role:	*Co-Investigator*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Behlau has worked closely with Dr. Kaplan to complete the aims of the project, particularly focusing on the in vivo aims.*

Funding Support:	*This grant*

Name:	*Chiara Ghezzi*
Project Role:	*Research assistant professor*
Researcher Identifier (e.g. ORCID ID):	*https://orcid.org/0000-0002-9800-4397*
Nearest person month worked:	4
Contribution to Project:	*Dr. Ghezzi had contributed to the design and the development of the silk device and drug delivery system, as well as supervising all the aspects of this project.*
Funding Support:	*This grant*

Name:	*Ricardo Louzada*
Project Role:	*Surgeon*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Louzada had contributed in performing the animal surgeries.*
Funding Support:	*Tufts Medical Center*

Name:	*Jay Duker*
Project Role:	*Collaborator*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Duker had supervised all the surgical aspects of the animal work.*
Funding Support:	*Tufts Medical Center*

Name:	*David Kaplan*
Project Role:	*Principal Investigator*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Kaplan had supervised all aspects of the project.*
Funding Support:	*Tufts University*

Special Reporting Requirements

Appendices: